

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No. L222

Application to Food Analysis (No.20)

—Determination of Food Additives—

As examples of determination of food additives, analyses of antimolds (thiabendazole, diphenyl and *o*-phenylphenol) as well as analyses of preservatives (benzoic acid, sorbic acid and dehydroacetic acid) are introduced in Application News No.197, and simul-

taneous analysis of sweetenings (aspartame and saccharin) and analysis of an antioxidant (EDTA - 2Na) in No.L214. Introduced here are examples of analysis of phenolic antioxidants and preservatives (*p*-hydroxybenzoic acid esters).

■ Determination of Antioxidants (Phenol Derivatives)

Given here is a method for the determination of a number of the common antioxidants in edible fats and oils using a stepwise binary gradient elution.

The alkyl tails of the gallic acid esters, PG, OG and DG, make them lipophilic for easier solution in fats and oils, and they are known to be synergistic with the mono-butylated hydroxyanisoles (BHAs) and dibutylated hydroxytoluene (BHT), which are themselves synergists. The commercial

BHA products are usually mixtures of the isomers *2-tert*-BHA and *3-tert*-BHA, but the standard sample prepared for the separation shown here (Fig. 1) contained only the *ortho* (*2-*) substituted isomer (Peak 4).

The sample also contained the other substances shown and all are easily extracted from the oil or fat sample in the simple procedure given in Fig. 2.

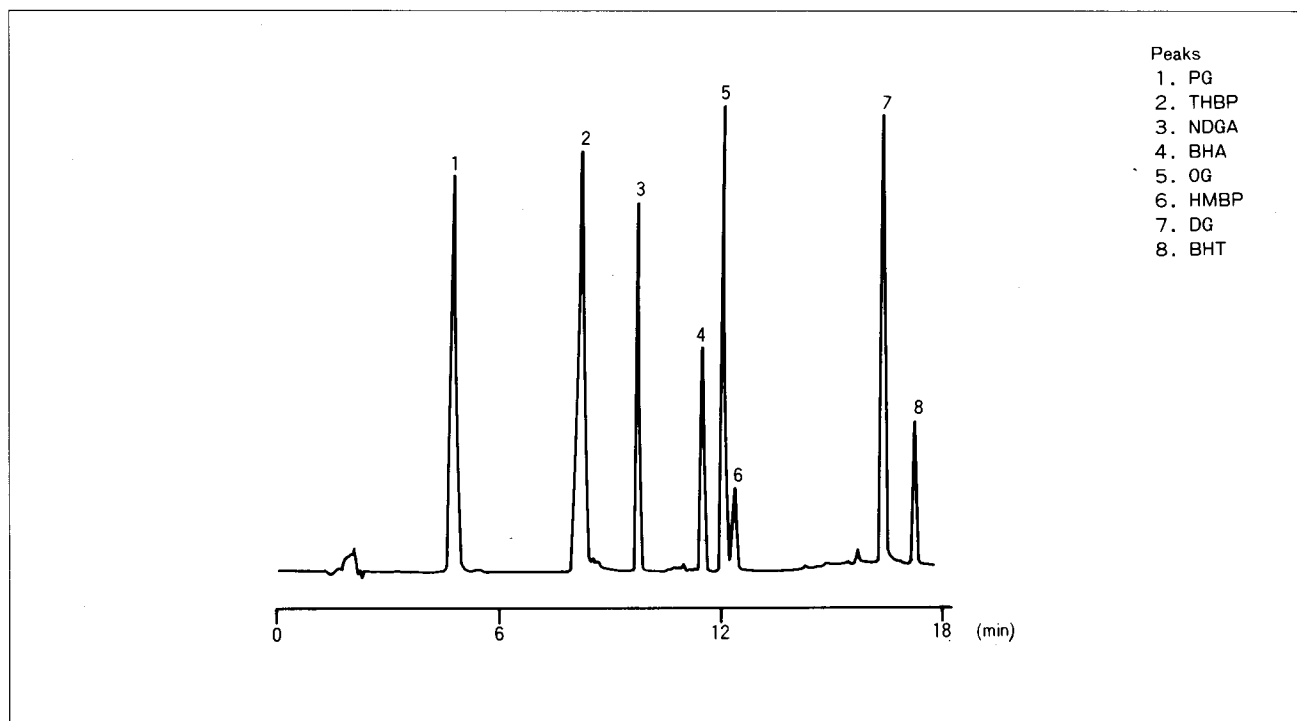


Fig. 1 Chromatogram of a Cooking Oil Extract (Antioxidants spiked)

Table 1 Analytical Conditions

Instrument	: Shimadzu LC-10A System
Sample	: Cooking Oil(ca.100ppm Spiked)
Column	: STR ODS- II (4.6mmI.D.×15cmL.)
Column Temp.	: 40°C
Mobile Phase	: A;10mM Sodium Phosphate Buffer(pH2.6) B;Acetonitrile
Gradient Elution	(Initial Condition BCONC 30%)
Flow Rate	: 1.0mL/min.
Detection	: SPD-10A, 280nm 2 AU/V, AT= 7

Gradient Program

TIME	FUNC	VALUE
4.00	BCONC	30
4.01	BCONC	63
10.00	BCONC	63
10.01	BCONC	90
16.00	BCONC	90
16.01	BCONC	30
25.00	STOP	

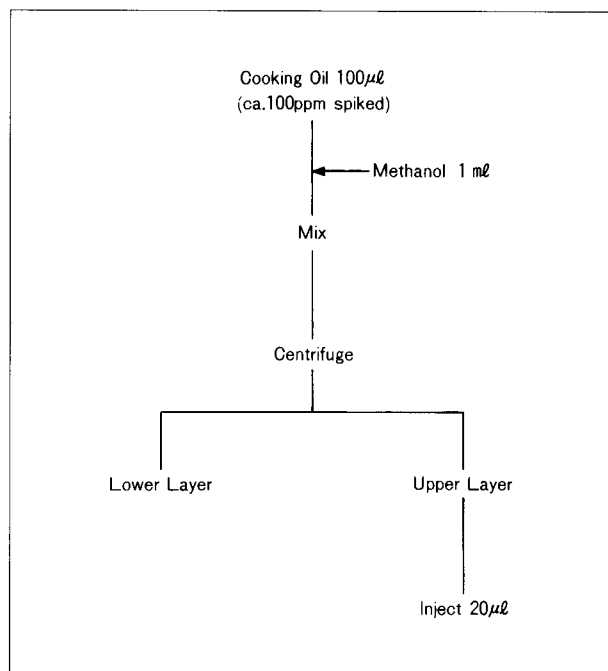


Fig. 2 Pretreatment Procedure

■ Determination of p-Hydroxybenzoic Acid Esters

A number of very similar substances to those mentioned earlier are the alkyl esters of p-hydroxybenzoic acid; the so-called *parabens*. The lower alkyl analogues, methyl through butyl, are widely used preservatives that appear to be multifunctional (e.g., fungicidal and antiseptic, etc.) and are much better tolerated by mammals.

The example separation shown in Fig. 3 is of the

parabens that were added (@ 100 ppm each) to a soy sauce which was diluted 10-fold with water. The dilute solution was then drawn through a disposable membrane filter into the syringe, the filter discarded, and 10ml of the filtrate was injected. Elution was from the same column as used for the phenolics, but with a simpler isocratic system, as given in Table 2.

Table 2 Analytical Conditions

Instrument	: Shimadzu LC-10A System
Sample	: Soy Sauce(ca.100ppm Spiked)
Column	: STR ODS- II (4.6mmI.D.×15cmL.)
Column Temp.	: 40°C
Mobile Phase	: 10mM Sodium Phosphate Buffer(pH2.6) /Methanol(1/1)
Flow Rate	: 1.5mL/min.
Detection	: SPD-10A, 270nm 2 AU/V, AT= 6

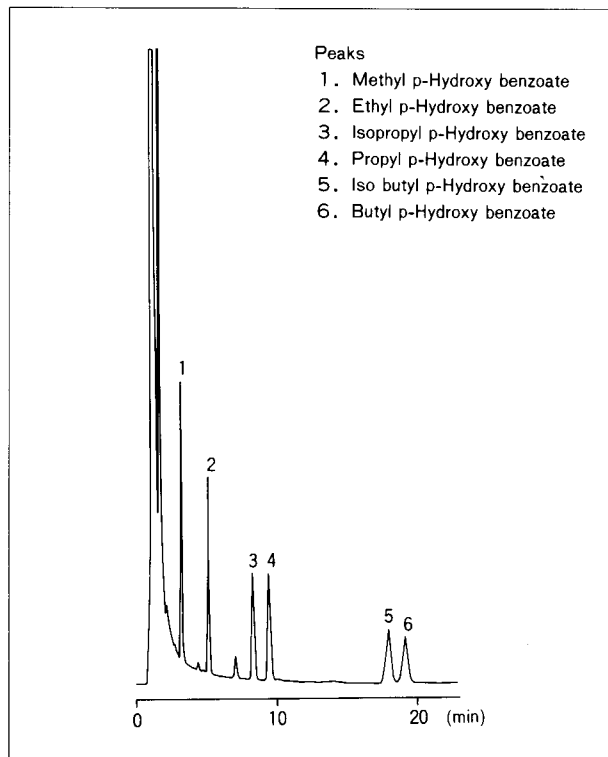


Fig. 3 Chromatogram of a Soy Sauce Extract (p-Hydroxybenzoic Acid Esters spiked)

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